# On the *Triturus vulgaris schreiberi* problem : electrophoretic data

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An electrophoretic study for genetic variation of a *Triturus vulgaris* population from Ravni Kotari, an area whose populations were named *T. v. schreiberi*, revealed that in contrast to previously analysed non-nominotypical subspecies, no gene loci showed especial allele frequency patterns This population is more similar to the nominotypical subspecies, which is congruent with their morphological similarities (SCHMIDTLER & SCHMIDTLER, 1983) and apparently denies a separate taxonomic position for the smooth newt from Ravni Kotari.

## INTRODUCTION

The smooth newt, *Triturus vulgaris*, underwent considerable evolutionary diversification in the Balkan region. A substantial morphological and genetical differentiation, mainly of populations restricted to small and marginal areas of the species range, occurred during the series of Pleistocene glaciations (BOLKAY, 1928 ; STEWARD, 1969 ; KALEZIĆ, 1984). Not surprisingly, subspecific status has been attached to most of these groups of populations (e.g. FREYTAG, 1954 ; BRAME, 1967 ; THORN, 1969). One of the subspecies, *Triturus vulgaris schreiberi* (WOLTERSTORFF, 1914), was described on a population sample from Bokanjačko Blato, near the town of Zadar (fig. 1). According to the original description, the individuals of this subspecies are characterized by their small size, unspotted belly and by a low and straight dorsal crest. However, SCHMIDTLER & SCHMIDTLER (1983), who studied morphological differentiation of the smooth newt populations inhabiting the Adriatic coast zone, denied the existence of *T. v. schreiberi*. They considered that the populations from Bokanjac (a village nearby Bokanjačko Blato) and the surrounding area (Ravni Kotari) belong to the nominotypical subspecies.

It has been revealed that smooth newt subspecies from Yugoslavia are characterized by different allele frequency patterns at some gene loci (KALEZIĆ, 1984 ; KALEZIĆ & TUCIĆ, 1984). Therefore, in order to yield additional insight into the taxonomic status of the Ravni Kotari smooth newt populations, we made an electrophoretic study for genetic variation of a population from the area in question.
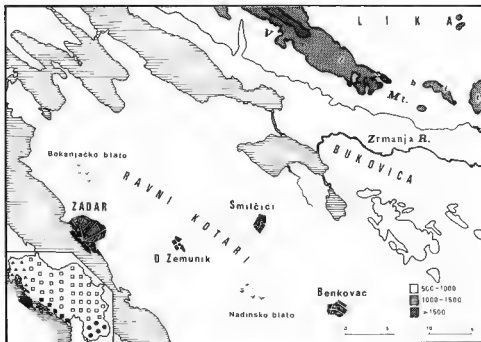
Fig 1. Map showing collection locality (Smilčići) and distribution of *Triturus vulgaris* subspecies in Yugoslavia □ -
*T. v. vulgaris*, ▲ - *T. v. meridionalis*, ■ - *T. v. dalmaticus*, ● - *T. v. graecus*.

## MATERIAL AND METHOD

The sample used for electrophoretic analysis was obtained from a population in the vicinity of the village of Smilčići, 20 km northeast of Zadar (fig. 1). Adult newts, 37 in number, were collected from a few holes dug nearby which are used as water supply sites for agricultural purposes. Interestingly, 23 individuals were paedomorphic (mainly females) and this again conformed to the statement that the Submediterranean area is an important centre of the smooth newt paedomorphosis (KALEZIĆ & DŽUKIĆ, 1986).

The electrophoretic techniques used to study protein polymorphism are thoroughly described elsewhere (HEDGECOCK, 1976). Twenty-one presumptive gene loci were scored (see Table I in KALEZIĆ, 1983). Genetic interpretation of allozymic data are based on criteria elaborated for *Triturus* newts by KALEZIĆ & HEDGECOCK (1980) and KALEZIĆ & TUCIĆ (1984). Estimates of genetic identity are expressed in term of NEI's (1972) *I* value.

## RESULTS

The following gene loci were monomorphic in the smooth newt population analysed : *Acph-2, Est-1, Fum, Gdh, G-6Pdh, Idh, Ldh, Pgi, 6-Pgdh, Pgm, Prot-1, Prot-2, Prot-3*, and

Table I. - Allele frequencies at four loci in the Smilčići sample, and weighted means of allele frequencies at the same loci of four *Triturus vulgaris* subspecies (from KALEZIĆ, 1984).

| Locus | Alleles | Smilčići | *T.v. vulgaris* | *T.v. meridionalis* | *T.v. dalmaticus* | *T.v. graecus* |
|-------|---------|----------|-----------------|----------------------|--------------------|-----------------|
| Acph-2 | 95 | 0.000 | 0.075 | 0.044 | 0.753 | 0.000 |
|        | 100 | 1.000 | 0.889 | 0.144 | 0.000 | 0.073 |
|        | 105 | 0.000 | 0.036 | 0.812 | 0.247 | 0.927 |
| Est-4 | 96 | 0.435 | 0.137 | 0.134 | 0.000 | 0.000 |
|       | 100 | 0.565 | 0.863 | 0.866 | 0.000 | 1.000 |
|       | 110 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| Mdh-2 | 93 | 0.056 | 0.186 | 0.044 | 1.000 | 0.870 |
|       | 100 | 0.944 | 0.637 | 0.889 | 0.000 | 0.130 |
|       | 107 | 0.000 | 0.177 | 0.067 | 0.000 | 0.000 |
| Me | 98 | 0.042 | 0.015 | 0.015 | 0.242 | 0.149 |
|    | 99 | 0.250 | 0.307 | 0.116 | 0.459 | 0.461 |
|    | 100 | 0.653 | 0.486 | 0.756 | 0.299 | 0.373 |
|    | 102 | 0.056 | 0.192 | 0.113 | 0.000 | 0.017 |

*Sod* (for abbreviations see Table I in KALEZIĆ, 1983). Polymorphic loci which were the most variable included *Est-4* with an observed heterozygosity of 0.484, *Me* (0.444) and *α-Gpdh* (0.300). The population from Smilčići has a mean heterozygosity per individual of $0.062 \pm 0.002$, with averaged number of alleles detected per locus being $1.47 \pm 0.04$. Metamorphic individuals appeared to have slightly more genetic variation than paedomorphic ones ; heterozygosity per individual was 0.068 and 0.059, respectively. Genetically these two subsamples ("paedomorphic" and "metamorphic") are very similar ; NEI's *I* value is 0.992.

In order to compare the overall genetic identity between the population in question and populations of smooth newt subspecies which have been electrophoretically analysed so far, we calculated the *I* values. NEI's measure was computed using weighted means of allele frequencies of *T. v. vulgaris*, *T. v. meridionalis*, *T. v. dalmaticus* and *T. v. graecus* (presented in Table I in KALEZIĆ, 1984). The population from Smilčići is genetically more similar to the nominotypical subspecies (I=0.987), than to *T. v. meridionalis* (I=0.977), *T. v. graecus* (I=0.907) and *T. v. dalmaticus* (I=0.865). For the same purpose, Table I shows allele frequencies of four gene loci for the Smilčići population as well as for the above-mentioned subspecies. These loci appeared to differentiate smooth newt subspecies which inhabit the Yugoslav part of the species range.

DISCUSSION

In contrast to previously analysed *T. vulgaris* subspecies, none of the twenty-one assayed gene loci of the population from Smilčići showed special allele frequency patterns. The

absence of a relatively specific genetic entity apparently cannot candidate the smooth newt population from Ravni Kotari as belonging to a different taxon. Allele frequencies of *T. v. vulgaris* populations are the most similar to those of the population we studied, which is congruent with their morphological similarities as suggested by SCHMIDTLER & SCHMIDTLER (1983).

A rather broad zone of gene flow between *T. v. vulgaris* and *T. v. meridionalis*, presumably secondary, was found (see fig. 3 in KALEZIĆ, 1984). Populations from the zone had *Acph-2*$^{100}$ and *Acph-2*$^{105}$ alleles in appreciable frequencies, while allele *Acph-2*$^{100}$ was by far the most common in *T. v. vulgaris* and allele *Acph-2*$^{105}$ was so in *T. v. meridionalis* (Table I). In the population from Smilčići the *Acph-2*$^{100}$ allele is fixed and this indicates that smooth newt populations from Ravni Kotari have no contact with nearby populations of *T. v. meridionalis* from Lika (see fig. 1). It was found that these groups of populations are separated by a zone which for unknown reason(s) contains only the Alpine newt (*Triturus alpestris*). Such a zone continues and also separates the Ravni Kotari populations from the nominotypical subspecies. In spite of isolation, the genetic structure of smooth newt populations from Ravni Kotari has not changed much from the main body of *T. v. vulgaris* populations. The relatively high level of genetic variability of the population in question might indicate a nonfragmented population structure of smooth newts from Ravni Kotari, and also that these populations have not suffered population size bottlenecks as has been suggested for some other populations of the Submediterranean zone (KALEZIĆ, 1984).

The population from Smilčići is substantially different from more southern subspecies, especially *T. v. dalmaticus* which comprises adjacent smooth newt populations (fig. 1). Electrophoretically, *T. v. dalmaticus* is well differentiated from the Smilčići population due to different alleles at loci *Acph-2* and *Est-4*, as well as substantial differences in allele frequencies at loci *Me* and *Mdh-2*.

## RÉSUMÉ

Une étude électrophorétique de 23 locus présumés de 37 individus d'une population de *Triturus vulgaris* de Ravni Kotari, région dont les populations ont reçu le nom de *T. v. schreiberi*, a montré que dans ce cas, contrairement aux autres sous-espèces non-nominatives étudiées jusqu'à présent, aucun locus ne manifeste de fréquence allélique particulière. Cette population est plus semblable à la sous-espèce nominative, ce qui est congruent avec les données morphologiques (SCHMIDTLER & SCHMIDTLER, 1983), et ce qui indique que les Tritons vulgaires de Ravni Kotari ne méritent apparemment pas une position taxinomique séparée.

*(Résumé traduit par A. DUBOIS)*

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